

Attorney Docket No: 4231/2002(Serial No.: 10/085,783)

Inventor: Liéw, et al.

Filed: February 28, 2002

Sequence Listing and Preliminary Amendment

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REMARKS

In addition to the "Sequence listing", Applicants further submit two copies of Table 1 on CDROM in ASCI format which was originally submitted on CDROM (in Excel format) at the time of filing of the above referenced patent application. No new matter is added.

Respectfully submitted,

Date:



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MARKED-UP VERSION OF AMENDMENTS:

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Please replace the paragraph starting at page 36, lines 17 through 29 and continuing on page 37, lines 1 through 2, with the paragraph below, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

From the amplified library, phage plaques are plated onto an appropriate medium.

Preferably, phage plaques are plated at a density of 200-500 pfu/150 mm plate onto an *Escherichia coli* XL1-blue MRF' lawn with IPTG/X-gal for color selection. The plaques are then randomly picked and positive inserts are identified by polymerase chain reaction (PCR), according to methods well known in the art and described hereinbelow.

Preferably, plaques are picked into 75 ul suspension media buffer (100 mM NaCl, 10 mM MgSO₄, 1 mM Tris, pH7.5, 0.02% gelatin). Phage elutes (5 ul) may be used for PCR reactions (50 ul total volume) with 125 umol/L of each dNTP (Pharmacia), 10 pmol each of modified T3 (5'- GCCAAGCTCGAAATTAAACCCTCACTAAAGGG-3') (SEQ ID NO: 58993) and T7 (5'-CCAGTGAATTGTAATACGACTCACTATAGGGCG-3') (SEQ ID NO: 58994) primers, and 2 U of Taq DNA polymerase (Pharmacia). Reactions are cycled in a DNA Thermal Cycler (Perkin-Elmer) [denaturation at 95°C for 5 minutes, followed by 30 cycles of amplification (94°C, 45 seconds; 55°C, 30 seconds; 72°C, 3 minutes) and a terminal isothermal extension (72°C, 3 minutes)]. Agarose gel electrophoresis is used to assess the presence and purity of inserts.

Please replace the paragraph starting at page 72, lines 22 through 26, and continuing on topage 73, lines 1 through 14, with the following paragraph:

From the amplified λ ZAP Express library, phage plaques were plated at a density of 200-500 pfu/150 mm plate onto *Escherichia coli* XL1-blue MRF' lawn with IPTG/X-gal

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for color selection. Plaques were picked into 75 ul suspension media buffer (100 mM NaCl, 10 mM MgSO₄, 1 mM Tris, pH7.5, 0.02% gelatin). Phage elutes (5 ul) were used for PCR reactions (50 ul total volume) with 125 umol/L of each dNTP (Pharmacia), 10 pmol each of modified T3 (5'- GCCAAGCTCGAAATTAAACCCTCACTAAAG GG-3') (SEQ ID NO: 58993) and T7

(5'-CCAGTGAATTGTAATACGACTCACTATAGGGCG-3') (SEQ ID NO: 58994) primers, and 2 U of Taq DNA polymerase (Pharmacia). Reactions were cycled in a DNA Thermal Cycler (Perkin-Elmer) [denaturation at 95°C for 5 minutes, followed by 30 cycles of amplification (94°C, 45 seconds; 55°C, 30 seconds; 72°C, 3 minutes) and a terminal isothermal extension (72°C, 3 minutes)]. Agarose gel electrophoresis was used to assess the presence and purity of inserts. PCR products are subjected to DNA sequencing reactions using specific primers, BigDye™ Terminator Cycle Sequencing v2.0 Ready Reaction (PE Biosystems), Tris MgCl buffer and water in a thermocycler. Sequencing reactions were incubated at 94°C for 2 minutes, followed by 25 cycles of 94°C, 30 seconds; 55°C, 20 seconds; and 72°C, 1 minute; and 15 cycles of 94°C, 30 seconds; and 72°C for 1 minute; and 72°C for 5 minutes. Reactions were then put on hold at 4°C until purified through methods well known in the prior art (i.e. column purification or alcohol precipitation). Automated sequencing was carried out with a PE Biosystems ABI Prism 3700 DNA Analyzer.